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NEW METHODS OF EVALUATION FOR COCKROACH REPELLENTS AND REPELLENCY OF ESSENTIAL OIL AGAINST GERMAN COCKROACH (Blattella germanica L.)

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/133[1]

Introduction

Recently, some cockroaches such as German cockroaches and black cockroaches have been increased as city type home harmful insects in general homes and restaurants and become large social problems in terms of [illegible] and sanitation. At present, methods for spraying insecticides such as organophosphoric acid ester group and synthetic pyrethroid group on sites where cockroaches live are adopted for their extermination. However, since these cockroaches live in the vicinity of our neighborhood, there is a limitation in the treatment of chemicals, and in actuality, their safe extermination is very difficult.

On the contrary, chemicals for aggressively repelling the cockroaches, that is, repellents in our living environments have been researched to be used for the extermination of the cockroaches. Up to now, as the repellents of the cockroaches, compounds such as N,N-diethyl-m-toluamide[1-4], 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural (MGK.R-11)[1-4], 2-hydroxyethyl-n-octylsulfide (MGK.R-874)[1-4], tert-butylsulfenyldimethylidthiocarbamate (MGK, R-55)[1-4,8], methyl-α-

1 Numbers in the margin indicate pagination in the foreign text.

cyano-β-butylheptanoate[3,8], cumen hydroperoxide9), N,N-diethylnoramide[7,12], and allyl caproate[10] are reported. However, any of these compounds exhibits considerable effects in each biological test method, however its repellency effect is weak for the use in a practical extermination of the cockroaches, and it can be said that there is no compound that can be practically provided in terms of odor, safety, duration, etc.

Accordingly, this author reviewed substances that had olfactory repellency equivalent to those of conventional compounds and could expect a spatial repellency effect in researching new repellent substances.

Also, conventional test methods, for example, filter paper cylinder method, shelter method[1], cylinder method[1], slanting card method[2], carton method[3], feeding method[4], etc., are /134 mainly used to evaluate a contact repellency, however this author researched simple evaluation methods of the olfactory repellency - "test tube method" and "beaker method" -. Next, noticing that the cockroaches exhibit a repellent behavior to some foods and spices, the repellency of various spices being added to foods, that is, natural essential oils against German cockroaches (Blatella germanica L.) was investigated using the above-mentioned methods. As a result, interesting discoveries were obtained, and they are reported here.

Experimental materials and methods

1. Insects provided to tests

German cockroaches (Blattella germanica L.) that were serially raised in the Biological Division of Japan Environmental Sanitation Center were used.

2. Compounds provided to tests

As comparative compounds, N,N-diethyl-m-toluamide, 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, 2-hydroxyethyl-n-octylsulfide, di-n-propylisocinchromeranate, α-naphthoquinone, p-dichlorobenzene, and naphthalene were used. The natural essential oils provided to tests were 87 species and show in Table V.

3. Adjustment of samples

In the test tube method, a paper disc in which the compounds provided to the test were treated with an acetone solution with a fixed concentration was used, and in the beaker method, 3 g heated melted solution of 2,4,6-triisopropyl-1,3,5-tricosane (sublimate carrier, made by Ogawa Spice K.K.) was put into a plastic container (2.4 cm in diameter and 1.3 cm in height), held, and formulated. A lid having an opening gate with a fixed surface area was covered on the container and used.

4. Testing methods

In the test tube method (Figure 1), a contaminated filter paper (will be mentioned later) attached with an excrement of German cockroaches at the bottom of a test tube (1.6 cm in diameter and 16.5 cm in length), and one male adult insect or female adult insect of the insects provided to the test or one ovary and spermary part of young insects after 2 days of the incubation was held in the test tube and lidded with an absorbent cotton. The test tube was held for one night and treated with 0.02 ml acetone solution of the compound with a fixed concentration provided to the test, and the paper disc (made by Toyo Filter Paper K.K., 0.8 cm in diameter and 0.1 cm in thickness) was held in the above-mentioned contaminated filter paper in the test tube. The test tube treated was laterally fallen, and the repellency effect was decided from the position of the cockroach in the test tube after 24 h according to Table I. An example was shown in Figure 2. Also, three series were adopted for one group, and the effective minimum concentration (minimum effective condensation of 100% repellency, hereinafter abbreviated to MCE_{100}) was attained by the concentration showing the repellency effect of (+) level in all three series.

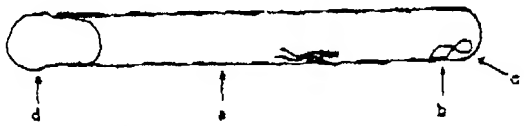

Fig. 1 Diagram of the test tube method for cockroach repellent test.
a: test tube (ϕ 1.6 cm × 16.5 cm), b: filter paper (ϕ 0.5 cm × 0.5 cm) treated with pheromone of cockroach, c: paper disc (ϕ 0.8 cm × 0.1 cm) treated with tested compound, d: absorbent cotton.

Table 1 Evaluation of repellency.

Response		Evaluation for repellency	Indication
Test tube method	Beaker method		
Aggregation on filter paper	Over 60 roaches in beaker	None	−
Location around middle position	59–30	Slight	±
Location at the end	29–5	Moderate	+
———	4–1	Strong	++
———	0	Remarkable	+++

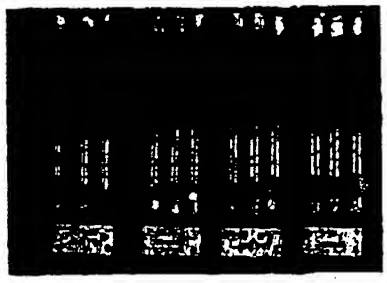

Fig. 2 Cockroach repellency of a sample (Japanese mint oil, 0.1 mg/disc, after 24 hr) by the test tube method.

In the beaker method (Figure 3), the inner surface wall/135 of a testing pot made of stainless steel (100 cm x 100 cm, 15 cm in height) was treated with grease, and a filter paper (made by Toyo Filter Paper K.K., No. 2, 15 cm in diameter) was placed on it. A plastic petri dish (9 cm in diameter and 1.5 cm in height) whose both side surfaces were open was fallen on it, and 1 l beaker (11 cm in diameter and 14.5 cm in height) was covered

on it. A fixed number of insects provided to the test (a group including male and female adult insects and young insects) was pastured in the above-mentioned pot. After a lapse of one night, the plastic container containing the sample stood still on the above-mentioned petri dish. Then, the cockroaches in the beaker were counted with time and compared with a non-treated group, and the existence of the repellency effect was decided as shown in Table I by the degree of reduction in the number of cockroaches. Three series for one group were adopted.

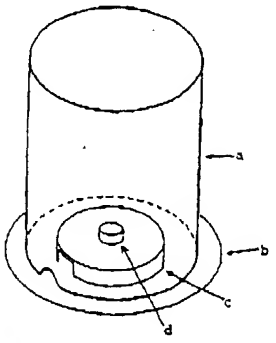

Fig. 3 Diagram of the beaker method for cockroach repellent test.
a: beaker (ϕ 11.0 cm × H 14.5 cm), b: filter paper (ϕ 15 cm), c: plastic laboratory dish (ϕ 9.0 cm × H 1.5 cm), d: sublimate containing the tested compound (ϕ 2.45 cm).

Results

1. Standardization of the contaminated filter paper being used in the test tube method

A filter paper (10 cm x 15 cm) was placed on the bottom face of a plastic raising cage (20 cm x 35 cm, 25 cm in height), and about 5,000 insects provided to the test (a group including

male and female adult insects and young insects) were pastured and fed with water and solid stuffs (made by Oriental Yeast Co., Ltd., for mice and rats). The above-mentioned filter paper contaminated with excrements of the insects provided to the test was called a "contaminated filter paper." In order to standardize the contaminated filter paper being used in the biological test, the effect of the contamination period in the cage on the repellency was compared. The contaminated filter paper was cut into a size of 0.5 cm x 0.5 cm and placed at the bottom of the test tube, and the aggregation rate of the tested insects in a state in which the samples were not included on the filter paper was investigated. As shown in Table II, the female adult insects exhibited a high aggregation rate of 90% or more without being related to the contamination period. On the contrary, the male adult insects and the male young insects of 2 days of age were respectively about 70% and about 65% in the contaminated filter paper of one week and respectively about 90% and about 75% in the contaminated filter paper of four weeks. The improvement of the aggregation rate was recognized by lengthening the contamination period. Next, using only the test tube in which the tested insects were aggregated on the contaminated filter paper, the effect of the contamination period on the MEC_{100} value of 2,3,4,5-bis(Δ^2-

butenylene)tetrahydrofurfural and spearmint oil (Scotch type) was investigated. As shown in Table III, in both two samples, in case the contaminated filter paper of one week was used, the MEC_{100} value was about 1/10 of the value of the case where the contaminated filter paper of three weeks or more was used, regardless of male and female adult insects and young insects. Also, for the contamination period of 3 weeks to 6 weeks, no meaningful difference was recognized. Therefore, a contaminated filter paper of 4 weeks was used in the subsequent biological tests.

Table 2 Effect of filter paper contaminated with roach excrement on aggregated activity using the test tube method

Contaminating time of filter paper (weeks)	% aggregation on the filter paper		
	Male	Female	Nymph
1	69	92	63
2	73	95	69
3	80	90	74
4	88	97	76
5	92	98	80
6	90	95	76

Male, Female; $n=100$, Nymph; $n=50$.

Table 3 Effect of filter paper contaminated with roach excrement on the evaluation of repellency using the test tube method.

Time contaminated with roach excrement (weeks)	Repellency MEC_{100} *) (10^{-n} mg/disc) n	
	Compounds tested	
	2-Hydroxyethyl-n-octylsulfide	Spearmint oil (Scotch type)
1	3	3
2	2-3	2-3
3	2	2-3
4	2	2-3
5	2	2-3
6	0	2-3

*) Minimum effective condensation of 100% repellency.

2. Repellency effect evaluation by the test tube method /136

As shown in Table IV, in α-naphthoquinone, 2-hydroxyethyl-n-octylsulfide, and naphthalene, there was no difference between the male and female adult insects and the young insects, and the repellency effect with a MEC_{100} value of 0.1-0.01 mg/disc was shown.

Table 4 Repellency of reference compounds for German cockroach.

No.	Compound	Repellency					
		Test tube method b)EC_{100} a)(10^{1-n} mg/disc) n			Beaker method b) Evaluation c) after		
		Male	Female	Nymph	5	24	170 (hr)
000	Untreated	−	−	−	−	−	−
001	Acetone	−	−	−	−	−	−
002	2,4,6-Triisopropyl-1,3-5-tricasane (sublimate carrier)	−	−	−	−	−	−
003	*N,N*-Diethyl-*m*-toluamide	1	1	1-2	−	−	−
004	2,3,4,5-Bis(Δ^2-butenylene)-tetrahydrofurfral	1-2	2-3	2-3	−	−	−
005	2-Hydroxyethyl-*n*-octylsulfide	2	2	2-3	−	±	±
006	Di-*n*-propylisocinchomeronate	0	0	0	−	−	−
007	α-Naphthoquinone	2-3	2-3	2-3	−	−	−
008	*p*-Dichlorobenzene	(dead)	1 (dead)	1 (dead)	−	−	−
009	Naphthalene	2	2	2	−	−	−

a) Minimum effective condensation of 100% repellency.
b) 2 w/w% Sublimate (carrier: 1,4,6-triisopropyl-1,3,5-tricosane).
c) −: No repellency, ±: Slight repellency, +: Moderate repellency, ++: Strong repellency, +++: Remarkable repellency.

On the contrary, in the 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, an equivalent titer was shown for the female adult insects and the young insects, however only a weak effect was shown for the male adult insects. On the other hand, in N,N-diethyl-m-toluamide, di-n-propylisocinchromeronate, and p-dichlorobenzene exhibited the repellency effect at only a high concentration of the MEC_{100} value of 1 mg/disc or more.

However, p-dichlorobenzene exhibited an insecticidal effect on the tested insects by the treatment of 1 mg/disc.

As shown in Table V, of natural essential oils, Japanese mint oil (No. 50) and spearmint oil (Nos. 85, 86, and 87) exhibited the repellency effect at the lowest concentration of the MEC_{100} value of 0.1-0.01 mg/disc. 11 kinds of essential oils of Birch oil (No. 19), cascarilla bark oil (No. 29), Japanese pepper oil (No. 51), marjoram oil wild Spanish (No. 60), nutmeg oil (No. 63), origanum oil (No. 68), palmarosa oil (No. 70), pepper mint oil (Nos. 74, 75, and 76), perilla oil (No. 77), rose oil (No. 81), savoury oil (NO. 84), tolu balsam oil (No. 93) exhibited a MEC_{100} value of about 0.1 mg/disc (in some of the essential oils, a difference was recognized in the male and female adult insects and the young insects) and had an activity slightly weaker than that of Japanese mint oil, etc. Also, in several kinds of essential oils such as clove oil (No. 36) (MEC_{100} value, female adult insects > male adult insects) and eucalyptus oil (No. 43) (male adult insects > female adult insects), a difference was recognized in the activity due to the male and female difference. However, in other natural essential oils such as citronella oil (No. 35), lavender oil (No. 55), rosemary oil (No. 82), pennyroyal oil (No. 73), and anis oil

(No. 15), the repellency effect was recognized at only a high concentration of the MEC_{100} value of 1 mg/disc or more.

Table 5 *Repellency of essential oils for German cockroach.*

No.	Essential oil	Repellency: Test tube method MEC_{100}[a] (10^{-n} mg/disc)			Repellency: Beaker method[c] Evaluation[d] after		
		Male	Female	Nymph	3	24	170 (hr)
010	Allspice oil	0	0	0	−	−	−
011	Almond oil bitter	0	0	0	−	−	−
012	Ambrette seed oil	0	0	0	−	−	−
013	Amyris oil	0	0	0	−	−	−
014	Angelica root oil	0	0	0	−	−	−
015	Anise oil	0	0	0	−	−	−
016	Basil oil	1–2	1	1–2	+	−	−
017	Bay leaf oil	1	1	1	−	+	+
018	Bergamot oil	0	0	0	−	−	−
019	Birch oil	2	2	2	+	++	++
020	Black pepper oil	1	0	0	−	−	−
021	Boronia absolute	0	0	0	−	−	−
022	Calamus oil	1	0	0	−	−	−
023	Camomile oil	1	1	1	+	+	−
024	Canagna oil	0	0	0	−	−	−
025	Capsicum oil	0	0	0	−	−	−
026	Caraway oil	1	0	1	±	−	−
027	Cardamon oil	1	1	1	−	−	−
028	Carrot seed oil	0	0	0	−	−	−
029	Cascarilla bark oil	2	2	2	+	++	±
030	Cassia cinnamon oil	1	1	1	−	−	−
031	Celery seed oil	1	0	0	+	+	−
032	Chamomile oil	0	0	0	−	−	−
033	Cinnamon bark oil	1	1	1	+	+	+
034	Cinnamon leaf oil	1	1	1	+	++	±
035	Citronella oil	1	1	1	+	++	−
036	Clove oil	2	1	1–2	±	++	+
037	Coriander oil	2	2	2	+	+	+
038	Costus oil	0	0	0	−	−	−
039	Cubeb oil	0	1	0	−	−	−
040	Cumin oil	1–2	1–2	1–2	−	+	+
041	Dill seed oil	0	0	0	−	−	−
042	Estragon oil	1	0	0	−	−	−
043	Eucalyptus oil	1	2	1–2	++	++	±
044	Fennel oil	1	0	0	−	−	−
045	Garlic oil	1–2	1	1	++	+	−
046	Geranium oil	1	1	1	+	+	+
047	Ginger oil	1	0	0	+	±	−
048	Grapefruit oil	0	0	0	−	−	−
049	Hop oil	0	0	0	−	−	−
050	Japanese mint oil	2–3	2–3	2	++	++	++
051	Japanese pepper oil	1–2	2	2	±	+	+
052	Juniper oil	0	0	0	−	−	−
053	Laurel leaf oil	0	0	0	−	−	−
054	Lavandin oil	0	0	0	−	−	−

(to be continued)

Table 5 (continued)

No.	Essential oil	Repellency: Test tube method MEC_{100}[a] (10^{-n} mg/disc)			Repellency: Beaker method[b] Evaluation[c] after		
		Male	Female	Nymph	3	24	170 (hr)
055	Lavender oil	0	0	0	−	−	−
056	Lemon oil	0	0	0	±	−	−
057	Lemongrass oil	0	0	0	−	−	−
058	Lime oil	1	1	1	±	−	−
059	Mandarin oil	1	1–2	1	±	+	−
060	Marjoram oil wild Spanish	2	3	1–2	##	##	+
061	Mustard seed oil	1	0	0	−	−	−
062	Mystle oil	0	0	0	−	−	−
063	Nutmeg oil	2	2	1	+	##	±
064	Olibanum oil	1	1	1	−	−	−
065	Olibanum resin	0	0	0	−	−	−
066	Onion oil	1	0	1	−	−	−
067	Orange oil bitter	0	0	0	−	−	−
068	Origanum oil	1–2	2	1–2	±	+	+
069	Orris root oil	0	0	0	−	−	−
070	Palmarosa oil	1–2	1–2	1–2	±	##	−
071	Patchooly oil	1	1	1	−	−	−
073	Pennyroyal oil	0	0	0	−	−	−
074	Pepper mint oil (madorus type)	2	2	2	##	##	+
075	Pepper mint oil (wimmet type)	2	2	2	##	##	+
076	Pepper mint oil (vilamett type)	2	2	2	+	##	+
077	Perilla oil	1–2	2	1–2	±	##	−
078	Peru balsam oil	1	1	1	−	±	−
079	Petitgrain oil	0	0	0	−	−	−
080	Pimenta leaf oil	1	0	0	−	−	−
081	Rose oil	1–2	2	1–2	±	##	+
082	Rosemary oil	1	1	0	−	−	−
083	Sage clary oil	1	1	1	±	−	−
084	Savoury oil	1–2	1–2	1	+	##	+
085	Spearmint oil (native type)	2–3	2–3	2	+	+	##
086	Spearmint oil (Scotch type)	2–3	2–3	2	+	##	##
087	Spearmint oil (midwest type)	2–3	2–3	2	##	##	##
088	Star anise oil	1	0	0	−	−	−
089	Swamp bay oil	1	0	0	−	−	−
090	Summer rue oil	0	0	0	−	−	−
091	Tangerine oil	0	0	0	−	−	−
092	Thyme oil	1	1	1	+	+	−
093	Tolu balsam oil	1–2	1–2	1	+	+	±
094	Vanilla extract	1	1	1	+	+	−
095	Wormwood oil	0	0	0	−	−	−
096	White pepper oil	1	0	1	−	−	−

a) Minimum effective condensation of 100% repellency.

b) 2 v/w% Sublimate (carrier: 2,4,6-triisopropyl-1,3,5-tricosane).

c) −: No repellency, ±: Slight repellency, +: Moderate repellency, ##: Strong repellency, ###: Remarkable repellency.

3. Relationship between the pasturage density and the repellency of the tested insects by the beaker method

100, 500, 1000, and 2000 pieces of tested insects were pastured in the testing pot made of stainless steel, and the effect of the pasturage density on the repellency was investigated. As the samples, 2% sublimate (a surface area of 4.52 cm^2) of 2-hydroxyethyl-n-octylsulfide, Japanese mint oil, and spearmint oil (Scotch type) was used.

As shown in Table VI, the 2-hydroxyethyl-n-octylsulfide exhibited a remarkable repellency effect (+++) after one week at a low density of 100 pieces/m^3, however a strong repellency effect (++) was not recognized, even after 1,000 h at a high density of 500 pieces/m^3 or more. On the contrary, the Japanese mint oil and the spearmint oil exhibited a remarkable repellency effect (+++) at a density of 100 pieces/m^3 and a high density of 2,000 pieces/m^3 after 1 h and 7-8 h, respectively. In any of the compounds, if the pasturage density was raised, the repellency effect tended to be lowered. Therefore, the pasturage /139 density was set to about 1,000 pieces/m^3 in the subsequent biological tests, and the tests were carried out.

// Insert Table VI //

4. Effect of the surface area of the sublimate formulation in the beaker method on the repellency

A plastic container containing 2% sublimate of Japanese mint oil, spearmint oil (Scotch type), and eucalyptus oil was covered with an upper lid with a surface area of 4.52, 1.00, 0.54, and 0.18 cm_2, and the effect of the surface area on the repellency was investigated. As shown in Table VII, although the cross section of the formulation was the same in any of the samples, the decrease of the repellency effect was recognized with the decrease of the surface area of the upper lid. Also, no repellency effect was recognized at a surface area of 8 cm^2 in any samples.

Table 6 Effect of roach population density on time required to attain remarkable repellency (卌) beaker method.

Population density (number/m²)	Time required to attain remarkable repellency (卌) (hr)		
	Compounds tested		
	2-Hydroxyethyl-n-octylsulfide	Japanese mint	Spearmint oil (Scotch type)
100	24×7	1	7–8
500	>24×30	3	24
1,000	>24×60	6–7	24×3
2,000	>24×30	24×2	24×5

Tested sample: 2 v/w% sublimate, surface area 4.52 cm².

Table 7 Effect of surface area of sublimate preparation on repellency beaker method.

Compound	Surface area (cm²)	Repellency area			
		3	24	24×4	24×7 (hr)
Japanese mint oil	4.52	卌	卌	卌	卌
	1.00	+	卌	卌	卌
	0.54	+	+	卌	卌
	0.18	−	−	−	−
Spearmint oil (Scotch type)	4.52	+	+	卌	卌
	1.00	+	+	+	卌
	0.54	+	+	+	卌
	0.18	−	−	−	−
Eucalyptus oil	4.52	卌	卌	−	−
	1.00	+	−	−	−
	0.54	+	−	−	−
	0.18	−	−	−	−

Population density: 1,000 roaches/m³.

5. Effect of the addition rate of the tested compounds in the beaker method on the repellency effect

Using 0.5, 1.0, 2.0, 4.0, and 8.0 v/w% sublimate (a surface area of 4.52 cm^2) of 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, 2-hydroxyethyl-n-octylsulfide, Japanese mint oil, spearmint oil (Scotch type), and eucalyptus oil, the effect of the addition rate of the tested compounds on the repellency was investigated at a pasturage density of about 1,000 pieces of tested insects/m^3. As shown in Table VIII, no reduction was recognized in the number of cockroaches in the beaker at an addition rate of 4% or less in the 2,3,4,5-bis(Δ^2-

butenylene)tetrahydrofurfural and 1% or less in the 2-hydroxyethyl-n-octylsulfide. Also, at an addition rate of 8%, although a slight reduction was recognized in the number of cockroaches, a remarkable repellency effect (+++) was not recognized. In the eucalyptus oil, as the addition rate increased, a fast-acting remarkable repellency effect was shown, however at an addition rate of 8%, only a short duration of /140 about 4 days was recognized. In the Japanese mint oil and the spearmint oil, if the addition rate was increased, a remarkable effect was recognized in the repellency effect, and at an addition rate of 4%, a remarkable repellency effect was recognized for about 4 weeks.

Table 8 Effect of concentration of tested compounds on repellency beaker method.

Compound	Concentration (v/w%)	Repellency after							
		1/8	1	4	7	14	21	28	35 (day)
2,3,4,5-Bis (2-butenylene)tetrahydrofurfral	0.5	−	−	−	−	−	−	−	−
	1.0	−	−	−	−	−	−	−	−
	2.0	−	−	−	−	−	−	−	−
	4.0	−	−	−	−	−	−	−	−
	8.0	−	±	+	+	+	+	+	+
2-Hydroxyethyl n-octylsulfide	0.5	−	−	−	−	−	−	−	−
	1.0	−	−	−	−	−	−	−	−
	2.0	−	±	±	±	±	±	±	−
	4.0	−	±	±	+	+	+	+	+
	8.0	−	±	+	+	+	+	+	+
Japanese mint oil	0.5	±	±	±	±	+	+	−	−
	1.0	±	±	±	±	+	+	−	−
	2.0	±	±	±	±	+	±	+	−
	4.0	±	±	±	±	±	±	+	+
	8.0	±	±	±	±	±	±	±	±
Spearmint oil (Scotch type)	0.5	+	+	±	±	±	±	+	+
	1.0	+	±	±	±	±	±	+	+
	2.0	+	±	±	±	±	±	±	±
	4.0	+	±	±	±	±	±	±	±
	8.0	±	±	±	±	±	±	±	±
Eucalyptus oil	0.5	+	−	−	−	−	−	−	−
	1.0	±	+	−	−	−	−	−	−
	2.0	±	±	±	−	−	−	−	−
	4.0	±	±	+	−	−	−	−	−
	8.0	±	±	±	−	−	−	−	−

Population density: 1,000 roaches/m³.

6. Repellency effect evaluation by the beaker method

2% sublimate formulation (a surface area of 4.52 cm^2) of the tested compounds at a pasturage density of about 1,000 pieces of tested insects/m^3 was evaluated, and the results were shown in Table V.

Also, in this testing method, generally, the following repellency behaviors of the cockroaches were observed in the samples having a strong olfactory repellency effect. In other words, the sublimate formulation stood still on a petri dish, and the cockroaches in the vicinity of the inlet of the beaker were rapidly escaped to the beaker outside. Also, the cockroaches remote from the inlet were separated from the formulation, and a chewing operation of the feeler and the leg part by the mouth part was recognized at sites separated from the beaker. In this paper, the former is called an expulsion effect, and the latter is called a penetration prevention effect.

First, as shown in Table IV and Figure 4, in the 2-hydroxyethyl-n-octylsulfide of the comparative compounds, a mild reduction was recognized in the number of cockroaches in the beaker, and a weak repellency effect (±) was recognized. However, in other comparative compounds such as α-naphthoquinone, neither remarkable expulsion effect nor

penetration prevention effect was recognized, and the increase was recognized in the number of cockroaches in the beaker.

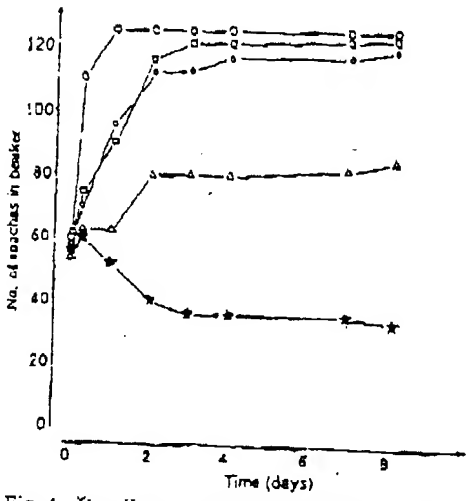

Fig. 4 Repellency curves of reference compound.
○: untreated, □: *N,N*-diethyl-*m*-toloamide, ◇: α-naphthalene, △: 2,3,4,5-bis(Δ^1-butenylene)-tetrahydrofurfral, ★: 2-hydroxyethyl-*n*-octylsulfide.

On the other hand, in the natural essential oils, as shown in Table V and Figure 5, the Japanese mint oil (No. 50) and the spearmint oil (native type and Scotch type Nos. 85 and 86) exhibited the best results similarly to the results in the /141 test tube method. In particular, the former is fast-acting and durable, and the latter exhibited a remarkable repellency effect (+++), though there was no fact-acting property. In 11 kinds of essential oils of Nutmeg oil (No. 63), peppermint oil (Nos. 74, 75, and 76), cinnamon leaf oil (No. 34), clove oil (No. 36), eucalyptus oil (No. 43), garlic oil (No. 45), marjoram oil wild Spanish (No. 60), onion oil (No. 66), palmarosa oil (No. 70),

perilla oil (No. 77), and savoury oil (No. 84), though the durability was slightly deficient, a fast-acting remarkable repellency effect (+++) was recognized. In 5 kinds of essential oils of Bay leaf oil (No. 17), ciannomon buck oil (No. 33), coriander oil (No. 37), cumin oil (No. 40), geranium oil (No. 46), Japanese pepper oil (No. 51), origanum oil (No. 68), and rose oil (No. 81), though there was no fast-acting property, a durable repellency effect (+) was recognized. However, a strong repellency effect was recognized in other natural essential oils such as citronella oil (No. 35).

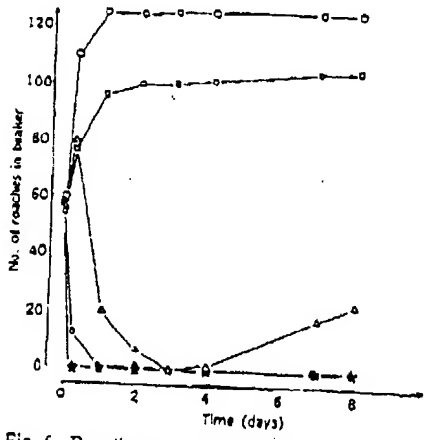

Fig. 5 Repellency curves of essential oils. ○: untreated, □: alice oil, △: bay leaf oil, ○: spearmint oil (Scotch type), ★: Japanese mint oil.

Consideration

1. Test tube method

This method is a repellency effect evaluation method using the property in which German cockroaches are aggregated on its excrements. As shown in the result 1, it is considered that if about 5,000 pieces of cockroaches are held in a cage, a contaminated filter paper showing a nearly fixed aggregation characteristic can be obtained from the repellency result.

From the result 2, it is considered that since the repellency effect can be expressed by the MEC_{100} value for each of male and female adult insects and young insects of the German cockroaches, the degree of repellency of the tested compounds due to the sex, growth stage, and individual difference can be investigated. Also, it is considered that a number of compounds can be tested under almost the same conditions, without considering a mutual effect of the tested compounds during the experiment.

However, the space of the test tube used is narrow, and in the compounds with a strong contact repellency such as α-naphthoquinone, it was recognized that the cockroaches were positioned at the opposite side of the paper disc after one day by repeating its contact repellency reaction. Therefore, it is considered that this method tests the olfactory repellency and the contact repellency. Also, in case this method is applied to cockroaches other than the German cockroaches, it might be

necessary to review separate inducing source, size of the test tube, etc.

2. Beaker method

This method is a method that considers modeling of a closed system space such as drawers and kitchen shelves where cockroaches live. In this method, the change of the number of cockroaches in a beaker is observed with time, and the olfactory repellency is tested as an expulsion effect and a penetration prevention effect from a fixed space. It is considered that any of the conventional methods such as carton method, shelter method, slanting card method, and feeding method is considered as a method that test a contact repellency effect due to the surface treatment of plates and sheets of tested compound rather than a method that tests the olfactory repellency effect. Therefore, it can be said that the conventional methods are excellent in the evaluation of spreading agents, however /142 they are inappropriate for the evaluation of vapor agents and aerosol agents starting with the sublimates mentioned in this paper. On the contrary, it is considered that this method has a feature capable of obtaining basic data for formulating the relationship between the addition rate and the repellency of the sublimate formulation, effective volume, effect durability,

effective concentration (Inazuka, unreported) in a gas, etc., as mentioned in the results 3, 4, and 5.

3. Evaluation on conventional cockroach repellents

The compounds of α-naphthoquinone, 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, 2-hydroxyethyl sulfide, and naphthalene exhibited the repellency effect at a low concentration in the test tube method. However, in the beaker method, only a weak olfactory repellency effect was shown in the sublimate formulation of 2-hydroxyethyl-n-octyl sulfide, and in other compounds, the effect showing an effective repellency was not obtained. The reasons for this are considered that the conventional repellents have a low vapor pressure and the concentration in a gas in the beaker in this sublimate formulation does not reach an effective concentration showing the repellency effect, or the olfactory repellency is not exhibited, though the contact repellency is exhibited.

In the 2-hydroxyethyl-n-octyl sulfide, 2% additive is used in 1 l beaker, and at this degree of effect, for the volume of drawers (an ordinary size of 10-20 l), though the population density is considered, the practicality as an olfactory repellent is deficient unless the surface area of the addition rate and the container is largely increased.

4. Evaluation on natural essential oils

From the overall results of the test tube method and the beaker methods, it is decided that the Japanese mint oil and the spearmint oil (native type and Scotch type) have a remarkable olfactory repellency effect, which does not exist in well-known repellent substances. Furthermore, in both the Japanese mint oil and the spearmint oil, the durability could not be expected in their raw solutions (Inazuka, unreported), however in the sublimate formulation using 2,4,6-triisopropyl-1,3,5-tricosane, a remarkable repellency effect was exhibited for about one month by the addition of 4%. It is considered that the reason for this is due to the vaporization suppression due to the retention effect of the sublimate carrier on the above-mentioned essential oil.

Also, the reasons why a fast-acting remarkable repellency was recognized in 11 kinds of essential oils such as Nutmeg oil are considered that the components related to the fast-acting property were rapidly evaporated and reached the minimum effective concentration or less or the affinity of the tested cockroaches to the effective components was caused. Also, the reason why a strong cockroach repellency was recognized in natural essential oils such as citronella oil, lavender oil, rosemary oil, and pennyroyal oil[10,11], which are reported that there is a strong repellency against mosquito, is that the

mutual action and the surface structure of chemical recipients for the repellent substances of mosquito and cockroaches are different. Therefore, the possibility that new discoveries and new repellents different from the correlation between the structure and the activity being obtained by the effect evaluation against mosquito are obtained. Also, in the test tube method, the fact that a strong repellency effect of several kinds of essential oils such as calamus oil against male adult cockroaches and cubeb oil against female adult cockroaches hints the difference in the susceptibility due to the difference in the sex of the cockroaches to certain effective components.

Based on the above considerations, in the future, the effective components of natural essential oils exhibiting a remarkable olfactory repellency will be isolated and identified, and the repellency of compounds in the vicinity of them will be investigated. Furthermore, the correlation between the structure and the activity of the repellents to cockroaches will be reviewed.

Conclusion

As new methods that can evaluate an olfactory repellency against German cockroaches, two methods of the test tube method and the beaker method were proposed. The test tube method is a

method that tests tested compounds in a test tube by the existence of the repellency reaction of cockroaches to a treated paper disc, utilizing a contaminated filter paper attached with excrements of German cockroaches as an inducing source. The beaker method is a method that treats samples containing tested compounds and tests the existence of spatial penetration prevention effect and expulsion effect by the change of the number of cockroaches in a beaker. Using these methods, well-known cockroach repellents and the olfactory repellency of natural essential oil were reviewed. The well-known repellents such as α-naphthoquinone, 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, 2-hydroxyethyl sulfide, and naphthalene exhibited the repellency effect at a low concentration in the test tube method. However, in the beaker method, only a weak olfactory repellency effect was shown in 2-hydroxyethyl-n-octyl sulfide, and an effective repellency was not recognized in other compounds.

On the other hand, in the natural essential oils, Japanese mint oil and spearmint oil (native type and Scotch type) exhibited a remarkable olfactory repellency. Also, a strong cockroach repellency was not recognized in the natural essential oils such as citronella oil which are reported that the repellency against mosquito is strong. The difference of the

repellency due to the sex was recognized in several kinds of essential oils such as calamus oil.

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Also, I thank Dr. Kazuyoshi Ogata of Japan Environment and Sanitation Center for useful advice and assistance.

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PTO 03-[5214] Japanese Article

KILLING ACTIVITIES OF THE VOLATILES EMITTED FROM ESSENTIAL OIS FOR DERMATOPHAGOIDES PTERONYSSINUS, DERMATOPHAGOIDES FARINAE, AND TYROPHAGUS PUTRESCENTIAE

[Seiyu No Kisan Seibun Niyoru Yakehyohidani, Konahyohidani, Oyobi Kenagakonadani Ni Taisuru Satsudani No Koka]

Fujio Watanabe, Shinichi Tadaki, Masatoshi Takaoka, Masazou Ishino, and Isao Morimoto

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Foreign Title	:	Seiyu No Kisan Seibun Niyoru Yakehyohidani, Konahyohidani, Oyobi Kenagakonadani Ni Taisuru Satsudani No Koka
Authors	:	Fujio Watanabe, Shinichi Tadaki, Masatoshi Takaoka, Masazou Ishino, and Isao Morimoto
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Source	:	Shoyakugaku Zasshi, 43(2), 163-168 (1989)

/163[1]

Introduction

A number of mites live inside houses. In particular, along with the change of house structures and house environments, mites of epidermic mites and Acaridae have recently tended to be abnormally increased. Among them, Dermatophagoides pteronyssinus and Deramtophagoides farinae of Chilean mites are important allergens related to allergic diseases. Also, it is said that 50-90% of patients with allergic diseases exhibit a susceptibility to the allergens of the mites, and these mites become serious problems in terms of public hygiene. However, extermination measures of these indoor mites are not currently established, yet.

In the extermination of the indoor mites, an organophosphoric acid ester group and a pyrethroid group insecticide are used, however their spray sites are inside the houses, and there is a problem in continuously spraying them over a long term in terms of safety of occupants. For this reason, in the extermination of the indoor mites, natural miticides with a high safety are preferably used.

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[1] Numbers in the margin indicate pagination in the foreign text.

In this paper, we invented a new simple miticidal testing method using indoor mites (Derematophagoides pteronyssinus, Dermatophagoides farinae, and Tyrophagus putrescentiae as insecticides being provided to tests in order to search for natural miticides with a safety higher than that of insecticides being currently used. Using this testing method, the miticidal effect of components (volatiles) with essential plant oil odors was investigated to exterminate the indoor mites. The essential plant oils have been widely used in foods, cosmetics, etc., and have a high safety, and the insecticidal effect of the odor components has been known. However, there are very few /164 reports[1)] in which the miticidal effect of the essential oils was investigated. Also, the inhibition effect on cholinesterase (ChE) activities having a close relationship with the insecticidal effect was measured for the essential oils exhibiting the miticidal effect, and the correlation with the miticidal effect was reviewed. The results are reported in this paper.

Experimental methods and materials

1. Mites being provided to tests and raising conditions

Dermatophagoides pteronyssinus (Dp), Deramatophagoides farinae (Df), and Tyrophagus putrescentiae (Tp) being serially

raised in our place were used. A medium in which a stuff (CE-2 made by Nippon Kurea K.K.) for experimental animals and a dry yeast (Ebios) were mixed at 1:1 was used. The medium was heated at 90°C for 1 h to prevent the admixture of mites other than the tested mites. The serially raised mites were put into an angular culture bottle containing the medium whose moisture content was adjusted to 16% and plugged (cellulose material, New Steri[transliteration] Plug) and raised in an incubator at a temperature of 25°C and a relative humidity of 75% (adjusted with a saturated sodium chloride solution). During the raising, the medium was mixed once or more per week. In the experiment, the mites whose multiplication state did not reach the climax (1-2 x 10^4 mites in 1 g medium) were used.

2. Essential oils and its main perfume components

82 kinds of essential plant oils used in the experiment are Abies oil, Almond Bitter oil, Ambrette Seed oil, Angclica oil, anis oil, Basil oil, Bay oil, Bergamot oil, Birch oil, Bois de Rose oil, Cajuput oil, Calamus oil, Camphor oil, Cananga oil, Caraway oil, Cardamon oil, Cassia oil, Cedarwood oil, Cedar leaf oil, Celery oil, Chamomile oil, Chenopodium oil, Cannamon Bark oil, Cinnamon Leaf oil, Citronella oil, Clove oil, Coriander oil, Costus oil, Cumin oil, Dill oil, Elemi oil, Estragen oil, Eucalyptus oil, Fennel oil, Gerandium oil, Grapefruit oil, Hiba

oil, HO oil, Ho Leaf oil, Japanese Red Pine oil (leaf), Jasmin oil, Jonquil oil, Juniper oil (bark), Laurel Leaf oil, Lavandin oil, Lavender oil, Lemon oil, Lime oil, Lovage oil, Mimosa oil, Mint oil, Myrrh oil, Myrtle oil, Neroli oil, Oak Moss oil, Ocotea oil, Olibanum oil, Opopanax oil, Orris oil, Parslcy oil, Patchouli oil, Pennyroyal oil, Perilla oil, Peru Balsam oil, Petitgrain oil, Pine oil, Pine Needle oil, Rose oil, Sandalwood oil, Spearmint oil, Sweet Orange oil, Taiwan Hinoki oil, Thyme oil, Turpentine oil, Vetiver oil, Wintergreen oil, Wormwood oil, and Ylang ylang oil. Among these essential oils, Japanese Larch oil, Japanese Red Pine oil, and Juniper oil were prepared from plants sampled by our company through a vapor distillation and received donations from Takasago Corporation and Cloister Chemicals Co.

Also, the main perfume components of the essential oils/165 used in the experiment and 22 kinds of its relative compounds are O-Anisaldehyde, Benzaldehyde, Citronellal, Cuminaldehyde, Perillaldehyde, Methyl Salicylate, Ethyl Salicylate, Propyl Salicylate, Methyl Benzoate, Ethyl Benzoate, Cincol, Citronellol, Geraniol, Linalool, Anethole, Safrole, p-Limonene, α-Phellandrene, β-Pinene, d-Carvone, l-Carvone, and Menthone. They were purchased from Tokyo Kasei Kogyo K.K. and Wako Pure Chemical Industries, Ltd.

3. Miticidal test

The mites used in the miticidal test of the essential oil were Dp, Df, and Tp, sampled with the medium from each angular culture bottle, and provided to the test.

In the test, an improved mite culture plate[2)] of Matsumoto et al. was used. In other words, a hole with a diameter of 15 cm was opened at the center of a transparent acryl resin plate (3 mm in thickness), and a Japanese paper with good air permeability was attached to the bottom. About 200 mites and the medium were put into the hole and covered with a slide glass (Figure 1). It was put into a glass petri dish with a diameter of 12 cm and a depth of 2.5 cm and put into a clock plate on which each test amount of essential oils (1, 2, 5, 10, 15, 20, 25, 30, 35, and 40 μl) was placed, lidded, and sealed with a para film (Figure 2). It was put into an incubator at a temperature of 25°C and a relative humidity of 75%, and the amount (μl) of essential oils required to kill all the mites (DL_{100}) in 24 h was attained. This test was carried out three times while changing the days under the same conditions for each sample. Also, the mites in the culture plate were observed in 48 h by a microscope after finishing the experiment, and the no motion of the mites was decided as the death of all the mites.

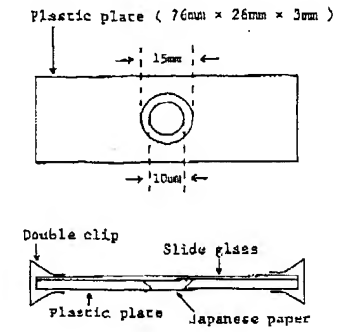

Fig. 1. The Shallow Container for Rearing the Mites with the Culture Medium

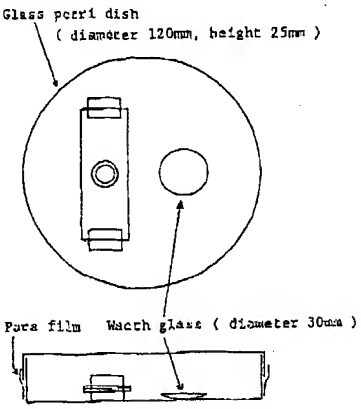

Fig. 2. The Vessel for the Test to Measure the 100% Mortality of the Mites

4. Cholinesterase (ChE) activity inhibition test

Ellman et al.' method[3] was adopted. 0.5 ml 25 mM butyrylthiocholine iodide solution and 0.5 ml 1 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) solution were added to a solution of 1.5 ml 67 mM phosphoric acid buffer solution (pH 7.4), 0.5 ml 0.05% essential oil solution (the essential oils were dissolved in 30% methanol), and 0.5 ml ChE (human serum, made by Sigma Co. (1 μg/ml) and reacted at 30°C for 20 min. Next, 0.5 ml 0.6 mM eserine solution was added to the reaction solution, and the reaction was stopped. Then, the absorbance As at 410 nm was measured. Instead of the essential oil solution, 0.5 ml 30% ethanol was added, and the absorbance of the control similarly operated was Ac. (1 - As/Ac) x 100 was assumed as the inhibition rate (%).

Results

The miticidal effect was seen in any of Dp, Df, and Tp for 52 kinds among 82 kinds of essential oils used in the experiment. Also, in the control that did not use the essential oils in this testing method, the motion of the mites after a lapse of 96 h from the completion of the experiment was compared with that of the experiment start. As a result, no change was recognized. Table I shows LD_{100} values of the essential oils in which the miticidal effect was seen. In Almond Bitter oil and Wintergreen oil, the DL_{100} value for Dp, Df, and Tp was 1 µl and exhibited a very strong miticidal effect. Also, Caraway oil, Dill oil, Ho oil, and Spearmint oil had a LD_{100} value of 2-5 µl for Dp, Df, and Tp and exhibited a strong miticidal effect. Bois de Rose oil, Cajuput oil, Cedar Leaf oil, Coriander oil, Cumin oil, Laurel oil, and Petitgrain oil had a LD_{100} value of 5-10 µl for Dp, Df, and Tp and exhibited a slight strong miticidal effect.

TABLE I. Killing Activity of the Essential Oils for *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Tyrophagus putrescentiae*

Name	LD_{100} Value (μl)[a]			% Inhibition of ChE activity	Name	LD_{100} Value (μl)[a]			% Inhibition of ChE activity
	Dp	Df	Tp			Dp	Df	Tp	
Abies Oil	30	30	20	0	Ho Leaf Oil	25	5	5	0
Almond Bitter Oil	1	1	1	20	Japanese Cedar Oil	>40	35	25	—
Angelica Oil	25	25	10	5	Japanese Larch Oil (Bark)	>40	>40	20	9
Anis Oil	>40	>40	10	0	Japanese Red Pine Oil (Bark)	>40	25	35	6
Basil Oil	20	20	5	4	Japanese Red Pine Oil (Leaf)	>40	>40	40	3
Bergamot Oil	20	15	10	10	Jasmin Oil	>40	10	10	11
Bois de Rose Oil	10	5	5	12	Laurel Leaf Oil	10	10	5	4
Cajuput Oil	10	10	5	6	Lavandin Oil	40	10	15	10
Camphor Oil	>40	15	30	20	Lemon Oil	25	10	10	—
Caraway Oil	5	2	5	6	Lime Oil	20	15	15	8
Cardamon Oil	25	15	10	15	Myrtle Oil	15	15	10	5
Cassia Oil	20	25	25	12	Neroli Oil	40	25	25	9
Cedar Leaf Oil	10	5	10	3	Ocotea Oil	>40	>40	10	23
Celery Oil	20	25	10	—	Olibanum Oil	40	15	15	0
Chamomile Oil	15	15	10	16	Perilla Oil	15	2	15	9
Cinnamon Bark Oil	>40	35	25	59	Petitgrain Oil	10	5	10	6
Citronella Oil	40	30	>40	16	Pine Oil	35	15	10	3
Coriander Oil	10	5	10	2	Pine Needle Oil	>40	15	10	6
Cumin Oil	10	5	10	6	Spearmint Oil	5	2	5	15
Dill Oil	5	5	5	2	Sweet Orange Oil	>40	10	10	29
Elleni Oil	25	30	10	4	Thyme Oil	>40	15	15	10
Estragon Oil	25	10	5	0	Turpentine Oil	>40	25	15	8
Eucalyptus Oil	15	10	10	5	Wintergreen Oil	1	1	1	0
Fennel Oil	>40	30	5	9	Wormwood Oil	30	10	10	5
Geranium Oil	>40	40	>40	9	Ylang ylang Oil	20	20	15	0
Grapefruit Oil	25	25	10	—					
Ho Oil	5	5	5	1					

[a] The amount of the essential oils required to give 100% mortality of the mites.

The results of the miticidal test of the main perfume components of the essential oils having a miticidal effect and 22 kinds of its relative compounds are shown in Table II. In aldehydes, Benzaldehyde (a main perfume component of Almond Bitter oil) and Cuminaldehyde (a main perfume component of Cumin oil) had a LD_{100} value for Dp, Df, and Tp of 1 μl and exhibited a very strong miticidal effect, and O-Anisaldehyde, Citronellal, and Perillaldehyde (a main perfume component of Perilla oil) also had a LD_{100} value for Dp, Df, and Tp of 1-5 μl and exhibited a strong miticidal effect. In esters, Methyl Salicylate (a main

perfume component of Wintergreen oil), Ethyl Salicylate, Methyl Benzoate, and Ethyl Benzoate had a LD_{100} value for Dp, Df, and Tp of 1-2 μl and exhibited a very strong miticidal effect. In chain-shaped terpene alcohols, Linalool (a main perfume component of Basil oil, Bergamot oil, Bois de Rose oil, Coriander oil, Ho oil, Ho Leaf oil, Lavandin oil, Neroli oil, Petitgrain oil, and Ylang ylang oil) had a LD_{100} value for Dp, Df, and Tp of 2-5 μl and exhibited a very strong miticidal effect, however Citronellol and Geraniol (main perfume components of Citronella oil, Geranium oil, and Rose oil) had little miticidal effect. In Safrole (a main perfume component of Ocotea oil) and Anethole (a main perfume component of Anis oil and Fennel oil) as phenols, there was no miticidal effect on Dp and Df, and the LD_{100} value for Tp was 10 μl and exhibited a slightly strong miticidal effect. d-Carvone (a main perfume component of Caraway oil and Dill oil) and l-Carvone (a main perfume component of Spearmint oil) as terpene group ring-shaped ketones had a LD_{100} value for Dp, Df, and Tp of 1 μl and exhibited a very strong miticidal effect.

TABLE II. Killing Activity of the Main Principles Containing in the Essential Oils and Its Analogous Compounds for *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Tyrophagus putrescentiae*

Name	LD_{100} Value (μl) a)			% Inhibition of ChE activity	Name	LD_{100} Value (μl) a)			% Inhibition of ChE activity
	Dp	Dp	Tp			Dp	Df	Tp	
O-Anisaldehyde	5	1	1	24	Citronellol	>40	>40	30	0
Benzaldehyde	1	1	1	28	Geraniol	>40	>40	30	0
Citronellal	5	2	5	9	Linalool	5	2	5	3
Cuminaldehyde	1	1	1	14	Anethole	>40	>40	10	8
Perillaldehyde	5	1	1	10	Safrole	>40	>40	10	—
Methyl Salicylate	1	1	1	0	D-Limonene	10	15	10	—
Ethyl Salicylate	1	1	2	2	α-Phellandrene	20	15	20	—
Propyl Salicylate	5	15	5	—	β-Pinene	20	25	10	—
Methyl Benzoate	1	2	1	0	*d*-Carvone	1	1	1	12
Ethyl Benzoate	1	2	1	—	*l*-Carvone	1	1	1	15
Cineol	5	10	10	—	Menthone	30	5	5	9

a) The amount of the samples required to give 100% mortality of the mites.

The difference in the susceptibility by the mite species to the essential oils is shown in Figure 3. In Dp and Df of Chilean mites, both of them showed approximate graph /167 patterns. However, judging from the number of essential oils having a miticidal effect, Df had a higher susceptibility to the essential oils than Dp. Also, in Tp of Acaridae, the number of essential oils with a LD_{100} value of 5-15 μl was very large, and the graph pattern showing the susceptibility exhibited an apparent difference from that of Dp and Df. From these facts, it was understood that the susceptibility to the essential oils was higher in Tp than Dp and Df. Tanaka et al.[4] report that the susceptibility to drugs is considerably different in Tyrophagus putrescentiae and Dermatophagoides farinae and the susceptibility of Tyrophagus putrescentiae is generally high. In this experiment, results similar to those were also obtained.

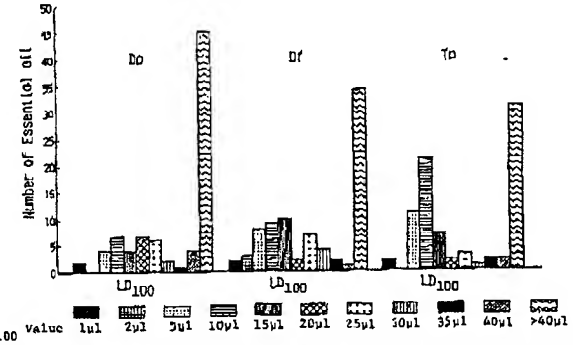

Fig. 3. Difference of Susceptibility to the Essential Oils among the Species of the Mites

It is known that the organophosphoric acid ester group insecticide exerts an insecticidal effect by inhibiting the ChE activity. When bees were killed by dropping parathion, ChE of the brain was inhibited by 90-94% of the normal state[5], and in nijuuyahoshidento[transliteration], it is reported[6] that 78% of the AChE activity is inhibited in a state in which all insects lie down after dosing TIA-230. Accordingly, the existence of the ChE activity inhibition effect in the essential oils, in which the miticidal effect was seen, and its main perfume components and relative compounds was investigated. As shown in Tables I and II, the ChE activity inhibition rate was in a range of 0-29% other than 59% in the Cinnamon Bark oil, and the ChE activity inhibition effect was small. Also, in the organophosphoric acid ester group insecticide, the size of the ChE activity inhibition effect is proportional to the insecticidal power. From this fact, the correlation between the ChE activity inhibition rate and the miticidal effect of the

essential oils, in which the miticidal effect was seen, and its main perfume components and relative compounds was investigated (Figure 4). In Dp, Df, and Tp, the ChE activity inhibition rate at each LD_{100} value was largely scattered, and the correlation between them was not seen.

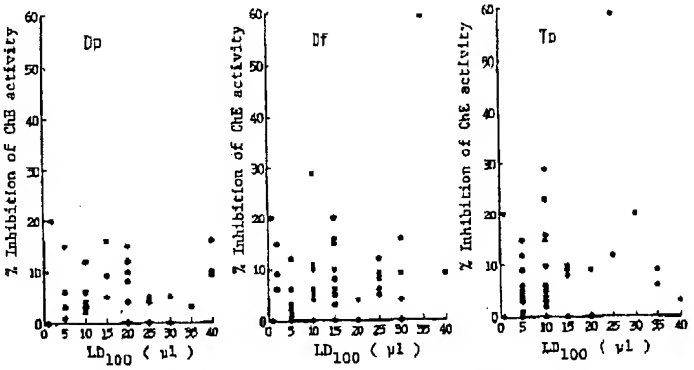

Fig. 4. Relation between the % Inhibition of ChE Activity and the Killing Activity in the Essential Oils

Consideration

The body length of the adult insects of epidermic mites and Acaridae is as fine as 0.3-0.4 mm, and a microscope is required for observing them. For this reason, Matsumoto et al.' mite medium plate was improved and used in the miticidal test. The mites in the culture medium can be directly observed from the outside by the microscope, and an air is circulated to some degree. At the same time, the mites are prevented from escaping from the gap of the vessel.

In testing the miticidal effect of drugs, a residue contact method and a medium mixture method[7] have frequently been used, however in these tests, since a filter or medium into which samples are immersed is dried, they are not suitable for the effectiveness test of odorous components (volatiles). In the miticidal test using the mite medium plate used in this study, since only the volatiles (inhaled poisons) is used in a sealed system, the effect on the mites is uniform, and the reproducibility is high. Furthermore, this vessel is small in scale and simple, and the mites in the culture plate can be directly observed by the microscope. For this reason, the miticidal effect of a number of drugs can be evaluated in a short time. Also, it is considered that this method can also be applied to other miticidal tests such as medium mixture method.

In the miticidal test of 82 kinds of essential oils, a very strong miticidal effect was seen in the Almond Bitter oil and the Wintergreen oil. Most of the aldehydes or esters such as benzaldehyde or methyl salicylate being included in the above-mentioned essential oils exhibited a strong miticidal effect in the main perfume components of the essential oils. It is considered that it is necessary to research the relationship between the chemical structure and the miticidal effect in them. Sato et al.[8] report that l-Carvone, Anethol, and Linalool have

an insecticidal effect on harmful insects of cloths and report that Lanalool also has an insecticidal effect on Tetranychiade and fleas[9]. In this experiment, l-Cavne and Linalool also exhibited a strong miticidal effect, and the miticidal effect was seen in the essential oils containing them. However, Anethole had a weak miticidal effect, and the essential oils containing it also had a weak miticidal effect. In this experiment, the strength of the miticidal effect of the main perfume components was often proportional to the strength of the miticidal effect of the essential oils containing them. Therefore, it is presume that the miticidal effect of the essential oils is mainly based on that of the main perfume components.

The organophosphoric acid ester group insecticide exerts an insecticidal effect by the ChE activity inhibition effect. Also, the size of the inhibition power appears as the strength of the insecticidal power. From this fact, the ChE activity inhibition effect of the essential oils was investigated, however the correlation was not seen between the strength of the miticidal effect and the size of the ChE activity inhibition effect. If EPN, parathion, malathion, etc., are oxidized in vivo, they exhibit a very strong ChE activity inhibition effect. In exerting the ChE activity inhibition effect of the essential

oils, the metabolic activation of an in vivo enzyme of the tested insects might be necessary. Also, it is known that lots of plants include polyphenols such as tannin and they nonspecifically inhibit the enzyme. In Cinnamon Bark oil in which the miticidal effect is weak, though the ChE activity inhibition effect is large, the relation of the polyphenols is considered.

From the results of the miticidal test of the essential oils, in consideration of the practicality as a miticide of the essential oils, when the essential oils have a strong perfume, though it is difficult, they are chemically stable, and the toxicity is also low. On the other hand, since this experiment is carried out in a closed system, the degree of effectiveness of their odorous components (volatiles) in houses in a highly open state is questionable. However, since it is considered that there are inhaled poisons and contact poisons in the odorous components of the essential oils, though they are used in similar formulations as those of the conventional insecticides, there is a miticidal effect. Also, since the amount of essential oils or main perfume components (Almond Bitter oil, Wintergreen oil, Benzaldehyde, Cuminaldehyde, Methyl Salicylate, d-Carvone, and l-Carvone) with a very strong miticidal effect is relatively small, their strong odors can

also be relaxed. Thus, it is considered that their practicality is considerably high.

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